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EXPERIMENT K-6-10

EFFECTS OF ZERO GRAVITY ON MYOFIBRIL PROTEIN CONTENT AND ISOMYOSIN DISTRIBUTION IN RODENT SKELETAL MUSCLE

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SUMMARY

The purpose of this experiment was to investigate the effects of 12 days of zero gravity (0G) exposure (Cosmos 1887 Biosputnik) on the enzymatic properties, protein content, and isomyosin distribution of the myofibril fraction of the slow-twitch vastus intermedius (VI) and the fast-twitch vastus lateralis (VL) muscles of adult male rats. Measurements were obtained on three experimental groups (n=5 each group) designated as flight-group (FG), vivarium-control (VC), and synchronous-control (SC). Body weight of the FG was significantly lower than the two control groups ($p < 0.05$). Compared to the two control groups, VI weight was lower by 23% ($p < 0.10$); whereas no such reduction was observed for the VL muscle. Myofibril yields (mg protein/g of muscle) in the VI were 35% lower in the FG compared to the controls ($p < 0.05$); whereas, no such pattern was apparent for the VL muscle. When myofibril yields were expressed on a muscle basis (mg/g x muscle weight), the loss of myofibril protein was more exaggerated and suggests that myofibril protein degradation is an early event in the muscle atrophy response to 0G. Analysis of myosin isoforms indicated that slow-myosin was the primary isoform lost in the calculated degradation of total myosin. No evidence of loss of the fast isomyosins was apparent for either muscle following space flight. Myofibril ATPase activity of the VI was increased in the FG compared to controls, which is consistent with the observation of preferential slow-myosin degradation. These data suggest that muscles containing a high percent of slow-twitch fibers undergo greater degrees of myofibril protein degradation than do muscles containing predominantly fast-twitch fibers in response to a relatively short period of 0G exposure, and the primary target appears to be the slow-myosin molecule.

INTRODUCTION

Previous findings on animals exposed to either zero gravity or to conditions of simulated non weight bearing activity such as hindlimb suspension clearly show that there is marked atrophy of hindlimb muscles comprised predominantly of slow-twitch fibers (Grindeland et al, 1985; Martin and Edgerton, 1985; Thomason et al, 1987a). Furthermore, Thomason et al (1987a; 1987b) reported that hindlimb suspension of rodents induced a preferential loss of myofibril protein in the atrophying slow-twitch soleus muscle. There was little evidence of a similar response occurring in the synergistic fast-twitch plantaris muscle (Tsika et al 1987d). This suggests that the myofibril fraction may be a target of protein degradative processes primarily in muscle fibers expressing slow-myosin when there is insufficient weight bearing activity placed on the muscle. The present study was undertaken to ascertain if a similar response occurs when animals are exposed to a zero gravity environment for sufficient duration to induce atrophy. Therefore, groups of rats were exposed to zero gravity during the Cosmos 1887 12 day mission which was launched in late September, 1987. The vastus intermedius, a knee extensor comprised largely of slow-myosin (Tsika et al 1987b) and the synergistic vastus lateralis, which is comprised chiefly of the fast myosin isoforms (Tsika et al 1987b), were examined in both flight and ground control groups for the following: a) muscle mass; b) myofibril protein concentration and ATPase specific activity; and c) estimates of absolute and relative isomyosin content. We tested the general hypothesis that zero gravity would induce 1) a preferential loss of slow myosin and a corresponding increase in myofibril ATPase activity in the vastus intermedius muscle and 2) minimal changes in the vastus lateralis muscle. The results reported herein are largely in support of this hypothesis.

METHOD

Experimental Design and Rodent Groups.

The muscles used for analyses in this study were obtained from animals selected for the Cosmos 1887 Biosputnik Flight. Adult male rats (n=5 each group) of the Czechoslovakian-Wistar strain were assigned initially to one of four experimental groups designated as: 1) Flight-group (FG); 2) Synchronous-control (SC); 3 Vivarium-control (VC); and 4) Basal-control (BC). In the present

study, for simplification in reporting the data and its interpretation, comparisons are reported for only the FG, SC, and VC groups, because the muscles from these latter groups were removed and processed at approximately the same time.

The FG (n=10 total assigned for flight) was housed in a single cage equipped with individual food nozzles and water lixits for each rat. Fourteen-gram boluses of food were administered at designated times each day so that each rat received a total of 55 grams of a paste diet. Water was provided ad libitum. The flight was launched on September 29, 1987 and returned 12 1/2 days later on October 12, 1987. The rats had their last meal on the final day of the flight and were not fed again for approximately for 42 hrs, at which time they were recovered from an alternate landing site (Siberia). Although the animals were without food, they appeared to have adequate water provision, since they did not drink water when it was provided at recovery. The animals appeared healthy and were warm. It is important to note that due to the alternate site landing, there was an approximate 48 hr delay between landing and sacrificing the flight animals at the designated site in Moscow.

The synchronous-control group (SC) was maintained in flight-type cages and fed a paste diet. They were exposed to the launch G force and vibration, deprived of food for 42 hours,andexposed to the same lighting regimen and temperature (23 deg. C) as flight rats after landing. After their simulated flight, sacrifice was delayed the same period as for the flight rats. The reentry G force and post flight transportation conditions of the flight animals were not mimicked for the SC group.

The vivarium-control group (VC) was kept in the same type of cage as the flight group. They were fed the same quantity of food per day but in only one feeding, and food was withdrawn 12 hrs prior to sacrifice. Post flight conditions were not mimicked for this group.

Tissue Sampling.

At the time of sacrifice the animals were weighed, and then specific organ components were removed including the muscles of the anterior thigh. On five animals from each experimental group, the vastus intermedius (VI) and the vastus lateralis (VL) muscles were removed, cleaned free of visible fat and connective tissue, weighed, and placed in vials containing 100% glycerol. The vials were stored at liquid nitrogen temperature, and they were eventually shipped by NASA to Irvine, California on dry ice in early November, 1987, at which time the biochemical analyses were begun.

Myofibril Extraction and Myofibril ATPase.

Myofibrils were prepared by the detergent treatment technique of Solaro et al (1971), as described in detail previously (Tsika et al, 1987a). After the final washing step, the myofibrils were suspended in 150 mM KCl-20 mM Imidazole (pH 7.0) and the concentration adjusted to 6 mg/ml with the use of the biuret protein assay (Gornall et al, 1949). Myofibril yields are reported as mg/gram and mg/muscle (table 2). This procedure is quantitative for the contractile proteins, because there is little evidence of loss of myosin in the washing process, based on electrophoretic analysis of the supernatants. Aliquots of the myofibril suspension were used immediately for ATPase activity (see below) and the remainder was prepared for storage (-20 deg. C) by suspending 1 volume of myofibril suspension with 2 volumes of a buffer consisting of 75% glycerol, 25 mM sodium pyrophosphate, 1 mM EGTA, and 0.5 mM 2-mercaptoethanol (pH 8.8).

Myofibril ATPase specific activity was determined at a free calcium concentration of ~10^{-4} M with the use of an EGTA buffer system as described in detail previously (Tsika et al, 1987a). Activity was expressed as micromoles of inorganic phosphate released per milligram of myofibril protein per minute (table 2).

Electrophoresis of myofibrils.

Aliquots of myofibrils suspended in the glycerol buffer were subjected to polyacrylamide electrophoresis to separate the native myosins according to the method of Hoh et al (1976). Briefly, native polyacrylamide gel electrophoresis was performed on 6-cm-long gels that were 4% in total acrylamide, 2.5% in bis(acrylamide) (expressed as a percentage of total acrylamide), 10% glycerol, 20 mM tetrasodium pyrophosphate (pH 8.8) at 4 deg. C. The gels were run in a Pharmacia GE-2/4 apparatus with recirculation of a running buffer consisting of 20 mM sodium pyrophosphate, 0.2 mM cystein, and 10% glycerol (pH 8.8). The electrophoresis apparatus was cooled by refrigeration at 4 deg. C and additionally by immersed cooling coils connected to an external recirculation refrigeration bath. The temperature of the recirculating buffer was maintained at -1 deg. C throughout electrophoresis. Gels were prerun at 90 V (15 V/cm) maintained constant for 30 min prior to sample application. Approximately 5 micrograms of protein were electrophoresed for 20 hrs at 90 V maintained constant. Gels were stained at the end of electrophoresis for two hrs with a solution that was 0.1% Coomassie Blue R-250, 30% isopropyl alcohol, and 10% glacial acetic acid. The gels were destained by diffusion in a solution that was 20% methanol and 10% glacial acetic acid.

Myofibril samples were also used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to the technique of Laemmli (1970) in order to estimate the relative percent of myosin heavy chain making up the myofibril protein pool. The myofibril suspensions were diluted two-fold with a buffer consisting of 100 mM Tris-HCl, 5% glycerol, 4% sodium dodecyl sulfate, 5% 2-mercaptoethanol, and 0.05% brom-phenol blue (pH 6.8) at room temperature. Purified myosin prepared from fast-twitch and slow twitch skeletal muscle were used as standards for identifying heavy chain and light chain proteins. The sample mixture was warmed to 100 deg. C for 2 min. Myofibrils were analyzed on 14% gels, which were run at room temperature in an apparatus with a central cooling reservoir through which tap water circulated. Electrophoresis was carried out at constant current (40 mA/slab gel) until the dye front reached the end of the gel. The gels were stained for 2 hrs at the end of the run using solutions as described above.

Quantitation of myosin isoforms and myosin heavy chain and light chain components.

Gel bands of the native myosins separated by pyrophosphate electrophoresis were analyzed densitometrically by directly scanning the gels at 630 nanometers using a Zeineh Soft Laser Scanning Densitometer (Biomed instruments Inc., Fullerton, Ca.) connected to an IBM PC equipped with the appropriate program for integration of peak areas. The relative proportion of myosin isoforms were obtained from the gel scan and corresponded to the percent area under the peaks of absorption of the isoform bands. Previously, we have identified five isomyosins in mixed rodent skeletal muscle in order of their decreasing ability to migrate into pyrophosphate gels as follows: fast myosins 1-3 (Fm_1, Fm_2, and Fm_3), intermediate myosin (Im), and slow-myosin (Sm) (Tsika et al, 1987b). These isomyosins have been fully characterized in terms of their light chain and heavy chain composition (Tsika et al, 1987b). In table 3, we present the distribution of these isomyosins in the vastus intermedius and vastus lateralis muscles of control and flight groups.

Myofibrils separated into denatured protein bands were also scanned on the Zeneih Densitometer in order to estimate the relative percent of myosin heavy chain making up the total myofibril protein. The assumptions and feasibility of this technique have been discussed in detail previously (Thomason et al, 1987a; Tsika et al, 1987c). With this procedure we have determined that the myosin heavy chain comprised 41$\pm$ 2 percent of the myofibril protein among all flight and control muscles analyzed. Also, there were no statistically significant differences among experimental groups (data not shown).

With the above information we were able to estimate the amount of both total myosin and the amount of individual isomyosins (mg/muscle) comprising a given muscle using the data as reported in tables 2 and 3 in combination with the following equation (1): mg myofibril protein/muscle X % myosin in myofibril pool X % isoform = estimated mg of myosin isoform/muscle. These data are reported in table 4.

Statistical analysis.

All values are expressed as the mean and standard error. Intergroup comparisons were made by using one-way analysis of variance. The significance of differences between groups was tested using a student t-test. Differences were considered significant at the 0.05 level of confidence.

RESULTS

Body and muscle weight.

Compared to control animals zero gravity induced a 21% reduction in VI muscle weight (table 1). However, this was not statistically significant due to the variability of response and the small number of observations. Little or no effect was observed for the VL muscle. In fact, muscle weights were larger for the FG as compared to the SC group. Body weight was significantly lower for the flight animals compared to the control groups. This can only be partially attributed to losses in muscle mass, because the majority of skeletal muscles in the rat are comprised of fast-twitch fibers, which do not appear to be as sensitive to zero gravity as muscles containing slow-twitch fibers.

Myofibril yields and myofibril ATPase activity.

Compared to both the VC and SC groups, zero gravity induced a significant reduction in th concentration of myofibril protein (mg/gram; $p<0.05$; table 2). The impact of this reduction is further seen when the myofibril protein data are expressed on a muscle basis (mg/g x muscle weight). In contrast, there was little evidence of an effect of zero gravity on the yield of myofibrils in the VL muscle.

Compared to the two control groups myofibril ATPase activity was significantly higher in the FG (table 2). This increase was attributed to a reduction in the relative amount of slow myosin comprising the VI of the FG, because slow-myosin has a lower ATPase specific activity than the faster isoforms. No pattern of a change in myofibril ATPase occurred for the VL muscle as a result of zero gravity exposure. However, there was poor agreement of VL ATPase activity among the SC and VC groups (table 2).

Myosin isoform distribution.

As shown in table 3, over 70% of the myosin expressed in the VI of control animals is in the Sm and Im forms. These isoforms are thought to have lower ATPase activity than the fast isoforms, which is evident by the ATPase data reported for the two muscles in table 2 . In contrast, the VL is comprised primarily of the fast isomyosin with less than 20% in either the IM or Sm form (table 3). Zero gravity induce a slight but non significant reduction in the relative content of Sm and a slight increase in the three fast isoforms in the VI muscle. In the VL muscle, there was an apparent shift suggesting a decrease in the relative content of Im and an increase in the relative content of Fm_3 isomyosin (table 3).

When the myosin content of the muscle (mg/muscle) is examined, it is apparent that there is a significant loss in the total myosin content of the VI muscle of the FG compared to the two control groups (table 4). This loss in total myosin in the VI muscle of the FG was attributed to reductions in the absolute amount of both the SM and Im isoforms. There is no evidence of a significant loss in the fast-myosins. This observation is further illustrated by the fact that there was little impact of zero gravity on the myosin isoform patterns in the VL muscle.

DISCUSSION

The most significant observation in this study is that after approximately two weeks of exposure to zero gravity there is a reduction in the capacity to maintain expression of the lower ATPase (and hence slower) isomyosins in certain skeletal muscles of rodents. Furthermore, the myofibril fraction as a total entity is a target for protein degradation processes in the absence of weight bearing activity. Analysis of the myosin content of the VI muscle indicates that the slow-myosin is the primary isoform that is degraded. This observation is consistent with previous findings on the soleus muscle of hindlimb suspended rats (Thomason et al, 1987a and 1987b), further suggesting that the absence of ground support activity is an important factor in inducing the atrophy response. Although there was a 42 hr delay in removing tissue from the flight animals upon return to normal gravitational conditions, which could have altered the magnitude of the response somewhat, we have observed previously on suspended animals that the half-life for regeneration of both the myofibril fraction and slow-myosin is approximately ten days (Thomason et al, 1987a). Thus, the delay in obtaining the tissue had a relatively small impact on reversing this response.

It is also interesting that although the vastus lateralis acts synergistically to the VI muscle, there was little evidence of either atrophy or degradation of the fast isomyosins in this fast-twitch muscle. However, it appeared that there was some loss of Im, which was balanced by an increase in Fm_3, thereby maintaining myofibril protein concentration and content in the VL muscle (table 2).

Analyses on myofibril ATPase activity suggest that there may be a net speeding of the VI muscle's contractile (force- velocity) properties following exposure to zero gravity, because there is a good relationship between myofibil ATPase activity and the shortening velocity of a given muscle (Barany 1967). This observation is consistent with the recent findings of Fitts et al (1986), which showed that the shortening velocity of the soleus. but not the superficial vastus lateralis, was increased following 14 days of hindlimb suspension.

If the above results on rodents apply to other mammalian species, including humans, it would appear that skeletal muscles expressing a large proportion of slow-twitch fibers (slow-myosin) are the chief target for the atrophy response that is associated with prolonged zero gravity exposure. Furthermore, in view of our recent findings suggesting an inability to significantly regenerate slow-myosin in large quantities in suspended rats that were subjected to low intensity treadmill running (Thomason et al, 1987b), it would appear that counter measures of sufficient duration requiring relatively high force output by those muscles sensitive to atrophy are necessary in order to maintain expression of the slow myosin and its associated myofibril proteins. Also, it is interesting that anabolic steroids, which could have an impact on maintaining muscle mass, are effective in conserving fast-twitch muscle but not slow-twitch muscle of suspended rats (Tsika et al, 1987d). Thus it would appear that mechanical stress factors, coupled with specific hormonal manipulations, may be necessary to provide sufficient counter measures to offset potential loss of all the myosin isoforms typically expressed in skeletal muscle when individuals are exposed to prolonged space flight.

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TABLE 1. BODY WEIGHT (GRAMS), MUSCLE WEIGHT (MILLIGRAMS), AND MUSCLE WEIGHT/BODY WEIGHT AMONG EXPERIMENTAL GROUPS.

Group	BW	VI	VL	VI/BW	VL/BW
Flight	303 ±3	189 ±44	892 ±63	0.623 ±0.14	2.94 ±0.19
Vivarium Control	342* ±9	242 ±30	964 ±37	0.720 ±0.10	2.81 ±0.05
Synchr. Control	349* ±7	246 ±35	827* ±57	0.700 ±0.10	2.37* ±0.16

Data are reported as mean ±SEM. * P< 0.05 flight vs control
VI=vastus intermedius; VL=vastus lateralis

TABLE 2. MYOFIBRIL PROTEIN YIELDS AND MYOFIBRIL ATPASE ACTIVITY IN MUSCLES OF EXPERIMENTAL GROUPS.

Group		Vastus Intermedius	
	mg/g	mg/muscle	ATPase
Flight	71 ±7	14.1 ±4.1	415 ±72
Vivarium Control	108 ±6*	26.1 ±3.2*	334 ±17*
Synchr. Control	107 ±3*	26.1 ±2.7*	342 ±32*
		Vastus Lateralis	
	mg/g	mg/muscle	ATPase
Flight	81 ±13	62 ±16	606 ±24
Vivarium Control	79 ±17	79 ±16	734 ±55
Synchr. Control	87 ±16	74 ±17	623 ±45

Data are mean ±SEM. ATPase is expressed as nMoles/mg myofibril protein/min.

* $P < 0.05$ flight vs control group

TABLE 3. RELATIVE PERCENT OF ISOMYOSINS EXPRESSED IN VASTUS INTERMEDIUS AND VASTUS LATERALIS MUSCLES OF EXPERIMENTAL GROUPS.

Vastus Intermedius

Group	Sm	Im	Fm_3	Fm_2	Fm_1
Flight	31.0 ±4.5	34.4 ±1.5	21.8 ±0.7	8.8 ±2.9	3.9 ±1.5
Vivarium Control	37.8 ±3.3	34.5 ±2.5	17.8 ±1.7	6.8 ±0.8	3.1 ±1.0
Synchr. Control	37.0 ±5.1	37.3 ±1.2	18.8 ±3.8	4.9 ±1.9	1.9 ±0.9

Vastus Lateralis

Group	Sm	Im	Fm_3	Fm_2	Fm_1
Flight	--	8.8 ±1.1	40.8 ±1.7	32.7 ±1.2	17.3 ±0.5
Vivarium Control	--	14.8* ±0.5	32.6* ±0.6	31.8 ±0.4	20.8 ±0.7
Synchr. Control	--	16.6* ±1.7	33.5* ±1.1	31.2 ±0.9	18.8 ±0.9

Data are mean ± SEM. Sm=slow-myosin; Im= intermediate- myosin; Fm=fast-myosin

* $P < 0.05$ control vs flight

TABLE 4. ESTIMATES OF TOTAL MYOSIN AND ISOMYOSIN CONTENT EXPRESSED AS MG/MUSCLE AMONG EXPERIMENTAL GROUPS.

		Vastus Intermedius		
Group	Total	Sm	Im	Fm
Flight	6.7 ±0.3	1.8 ±0.6	2.3 ±0.8	2.6 ±0.5
Vivarium Control	12.5* ±1.3	4.6* ±0.5	4.3* ±0.6	3.7 ±0.7
Synchr. Control	10.6* ±1.2	3.8* ±0.3	3.9* ±0.4	2.9 ±0.8

		Vastus Lateralis		
Group	Total	Sm	Im	Fm
Flight	31.1 ±5	--	2.6 ±0.4	28.4 ±5
Vivarium Control	31.0 ±6	--	5.4 ±2.0	25.7 ±5.0
Synchr. Control	29.6 ±6	--	4.4 ±0.9	24.0 ±4.0

Data are mean ±SEM. Sm=slow-myosin; Im=intermediate-myosin; Fm=fast-myosin.

* P< 0.05 flight vs control